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U.S. DEPARTMENT OF ENERGY | Office of Science

LIFE SCIENCES DIVISION E-NEWSLETTER

October, 2008

In this issue:

DOE scientific focus area notes

Low Dose Radiation Research

Life Scientists Well Represented at Annual Meeting of Environmental Mutagen Society
The 39th Annual Meeting of the Environmental Mutagen Society (EMS) was held October 18-22, 2008 in Puerto Rico. The meeting, whose theme was "Genes and the Environment: From Molecular Mechanisms to Risk", was organized and led by life scientists **Priscilla Cooper** and **Andrew Wyrobek** as 2008 Program Chair/President-Elect and EMS President, respectively. The conference served as a forum for integrating cutting-edge basic research in DNA repair, mechanisms of mutagenesis, and epigenetic regulation in response to environmental genotoxic agents (including low-dose ionizing radiation) with translational research, risk assessment, and regulatory concerns. Such cross-disciplinary integration has long been a unique strength of the EMS and is critically important for resolution of societal issues concerning evaluation of risk from environmental exposures to ionizing radiation and other genotoxins.

The Office of Science (BER) Low Dose Radiation Research program was a major sponsor of two symposia and a platform session in the emerging areas of epigenetic regulation and low dose radiation-induced genome and epigenome instability. These included presentations by life scientists **Stephen Ayers** (**Kohwi-Shigematsu** lab) and **Bo Hang**. Other major themes included effects of DNA damage on transcription; consequences of genotoxic damage to mitochondrial DNA; DNA damage in neurodegeneration, aging and cancer; insights into germline mutagenesis; DNA repair and damage responses; and environmental exposures and carcinogenic risk. Life Sciences Division scientists were prominent participants in these areas, with talks by **Altaf Sarker** and **Kelly Trego** from the Cooper lab, **John Tainer, Francesco Marchetti**, **Steven Yannone**, and by Wyrobek lab scientists **Aris Polyzos** and **Xiu Lowe**. In addition, **David Schild** chaired a session on mutagenic and carcinogenic mechanisms. During the meeting, LLNL senior scientist Larry Thompson, who has collaborated extensively with Schild, was presented with the EMS Award by President Wyrobek for his career-long contributions to understanding DNA repair mechanisms through somatic cell genetics.
Priscilla Cooper, 10/08

New Confocal Microscope for Low Dose Radiation Research
With BER Low Dose Radiation Research program funding, the Division has acquired the latest Zeiss LSM710 confocal microscope, which was installed on October 13, 2008. This instrument will be used to acquire quantitative image data for all projects in Berkeley Lab's low-dose Scientific Focus Area. The spectral detection, high sensitivity, and background suppression capabilities of this instrument enable high-resolution 3D multi-color imaging of cells in culture and tissues. Live cell incubation equipment allows dynamic studies over hours and days while photo-manipulation methods, such as FRAP, FRET, and RICS, enable analysis of protein dynamics, protein-protein interaction, and cell-cell signaling in live cells.
Damir Sudar, 10/08

Zeiss LSM710 confocal microscope

Low Dose Research Published in *Cancer Research*

From the authors: Radiation-induced genomic instability, in which the progeny of irradiated cells display a high frequency of nonclonal genomic damage, occurs at a frequency inconsistent with mutation. We investigated the mechanism of this nontargeted effect in human mammary epithelial cells (HMEC) exposed to low doses of radiation. We identified a centrosome-associated expression signature in irradiated HMEC and show here that centrosome deregulation occurs in the first cell cycle after irradiation, is dose dependent, and that viable daughters of these cells are genomically unstable as evidenced by spontaneous DNA damage, tetraploidy, and aneuploidy. Clonal analysis of genomic instability showed a threshold of >10 cGy [EDITOR'S NOTE: threshold for genomic instability (GI) is anywhere between 10 and 50 cGy]. Treatment with transforming growth factor beta1 (TGFbeta), which is implicated in regulation of genomic stability and is activated by radiation, reduced both the centrosome expression signature and centrosome aberrations in irradiated HMEC. Furthermore, TGFbeta inhibition significantly increased centrosome aberration frequency, tetraploidy, and aneuploidy in nonirradiated HMEC. Rather than preventing radiation-induced or spontaneous centrosome aberrations, TGFbeta selectively deleted unstable cells via p53-dependent apoptosis. Together, these studies show that radiation deregulates centrosome stability, which underlies genomic instability in normal human epithelial cells, and that this can be opposed by radiation-induced TGFbeta signaling.

Maxwell CA, Fleisch MC, **Costes SV**, Erickson AC, Boissière A, Gupta R, **Ravani SA**, **Parvin B**, Barcellos-Hoff MH. Targeted and nontargeted effects of ionizing radiation that impact genomic instability. *Cancer Research*, 2008 Oct 15;68(20):8304-11. PMID: 18922902

CG, 10/08

GTL-Genomics

CellulosicRoundtable.com Interviews Auer

In its ongoing series of Q&A's with leading cellulosic ethanol researchers, CellulosicRoundtable.com, a recently launched website covering the cellulosic ethanol sector, put Manfred Auer "Under the Microscope." The Q/A session with Auer on his research on cellulosic ethanol, a type of biofuel produced from lignocellulose, a structural material that comprises much of the mass of plants, can be found here: http://www.cellulosicroundtable.com/underthemicroscope.htm.

CG, 10/08

Nuclear Medicine

New Initiative in Nuclear Medicine

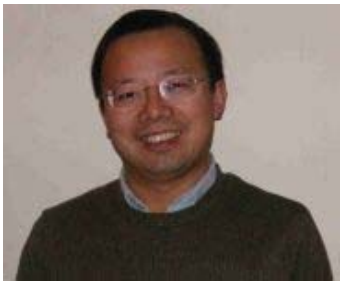
Through a DOE funded program on radio-labeling and imaging, the Life Sciences Division, in a multi-institutional effort, is developing a novel technology for *in vivo* imaging of gene expression. The technology will be optimized using fluorescent probes prior to the development of the more sensitive radiolabeled probes. While the fluorescently labeled probes are amenable to high throughput/spatial resolution for *in vitro* optimization, they can simultaneously be radio-labeled for higher sensitivity and for *in vivo* imaging. The technology enables continuous imaging of gene expression in living cells. For

example, (I) the changes in mRNA levels in response of environmental challenges will be visualized in the living organism. (II) In plant species, unlike mammalian cells, some mRNAs are transported from tissue to tissue via the phloem transport tubes. As a result, protein synthesis, in one cell-type, can occur using an mRNA template transported from another cell-type. Potential benefit will be better understanding of the communication mechanisms between tissues and organs in plants; therefore, developing means of controlling them through engineering of plants with desirable properties. (III) Similarly, applications of imaging gene expression, in mammalian species, may include (i) continuous monitoring of gene expression during embryonic development, and (ii) *in vivo* tracking of the location and differentiation state of stem cells during their progress through the body with a tracking machinery that utilizes much less cellular energy than current systems.

The technology pipeline utilizes fluorescently labeled ligands for characterizing toxicity, signal to noise ratio, and pharmacokinetics for cultured monolayer. Successful candidate ligands will screened further in a cultured multicellular system, which provides a substrate more similar to in vivo models. The program continues along two parallel tracks: (I) *targeting a specific mRNA* with allosteric aptamers that are activated upon hybridization with the mRNA and that produce a signal that is proportional to the amount of mRNA present. This effort is led by Marit Nilsen-Hamilton of Ames National Laboratory. (II) *Screening of ligands with desirable properties,* which include low toxicity, high signal to noise ratio, and optimum pharmacokinetics. In the second track, a team, led by Life Sciences **Bahram Parvin**, (i) has initiated screening of a large library of small molecule fluorescence ligands for their pharmacokinetic properties, (ii) is developing computational methods to associate chemical structure of ligands with their pharmacokinetic responses, and (iii) has extended their imaging bioinformatics system for chemoinformatics analysis. Several candidate ligands have been identified for a more detailed screening by live cell confocal microscopy. Once a candidate is identified, it will be attached to another molecule by Ames that will be recognized by the allosteric aptamers. Successful imaging of gene expression with fluorescently labeled probes will be followed by radio-labeling of ligands, at Berkeley Lab, for improved sensitivity and *in vivo* imaging.
Bahram Parvin, 10/08

Scientific news

Promising Geneticist Mao Joins the Division
The Life Sciences Division welcomes **Jian-Hua Mao** who joined the Cancer and Systems Biology Department as Geneticist Staff Scientist this month. Mao was identified as the most qualified candidate by a Search Committee of several life sciences Senior Scientists and chaired by **Joe Gray**, and his selection was reviewed and recommended by the Division Staff Committee.


Jian-Hua Mao

Mao holds a B.Sc. in Applied Mathematics of Southeast University, Nanjing, China, July 1986; a M.Medical Sc. in Biostatistics and Cancer Epidemiology, Bejing Medical University (now Peking University Health Science Center), Beijing, July 1989; and a Ph.D. in Radiation Oncology of the University of Glasgow, UK, July 1997. Since 1999 he has held several positions, such as Associate

Specialist, Specialist, Associate Researcher at the University of California, San Francisco and most recently an Assistant Adjunct Professor at the University of California, San Francisco.

Mao's major interest is to identify genetic networks and their functional polymorphisms controlling susceptibility to genomic instability and carcinogenesis induced by radiation, and he wants to pursue a systems biology approach to define the contribution of genetic diversity to the host in context of low dose irradiation. He wants to develop a comprehensive global view of the various sub-phenotypes that contribute to low dose radiation-induced cancer (e.g. inflammation, angiogenesis, genetic instability, cell cycle control, metabolism) by integrating the germline polymorphisms, somatic genetic events, gene expression changes and biological data. One of his research efforts will be to develop mouse models for the major forms of human cancer, which will enable him to identify genes that confer susceptibility or resistance to tumors induced by environmental mutagens and tumor promoters, such as radiation. His goal is to understand all stages of multi-step carcinogenesis in the mouse, in particular the relationships between germ line predisposition and somatic genetic changes in tumors. He believes that the identification of human homologues of these predisposition genes and discovery of their roles in carcinogenesis will ultimately be important for the development of methods for prediction of risk, diagnosis, prevention and therapy for human cancers.

His outstanding and pioneering research contributions have already made a significant impact in the national as well as in the international scientific community in his area of research interest. Some of his recent work was published in the September issue of *Science*.

Mao JH, Kim IJ, Wu D, Climent J, Kang HC, DelRosario R, Balmain A. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science,* 2008 Sep 12;321(5895):1499-502. PMID: 18787170.
CG, 10/08

Life Scientists Publish Article on Glioblastoma in *Nature*

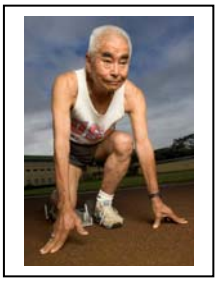
The NIH Berkeley Cancer Genome Center (BCGC), led by life scientists **Paul Spellman** and **Joe Gray,** played a major role in the "The Cancer Genome Atlas (TCGA) Research Network" article on glioblastoma published in the latest issue of *Nature*.

The goal of the TCGA project is to assess the value of large-scale multi-dimensional analysis of chromosomal aberrations, nucleotide substitutions and epigenetic modifications that drive malignant transformation in human cancers and to provide the data rapidly to the research community. The TCGA's first integrative study focuses on measurements of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas-the most common type of adult brain cancer-and nucleotide sequence aberrations in 91 of the 206 glioblastomas. This analysis provides new insights into the roles of ERBB2, NF1 and TP53, uncovers frequent mutations of the phosphatidylinositol-3-OH kinase regulatory subunit gene PIK3R1, and provides a network view of the pathways altered in the development of glioblastoma. Furthermore, integration of mutation, DNA methylation and clinical treatment data reveals a link between MGMT promoter methylation and a hypermutator phenotype consequent to mismatch repair deficiency in treated glioblastomas, an observation with potential clinical implications. Together, these findings establish the feasibility and power of TCGA, demonstrating that it can rapidly expand knowledge of the molecular basis of cancer.

The BCGC is one of seven Cancer Genome Characterization Centers funded by the NIH National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) as part of the Cancer Genome Anatomy Project (http://cgap.nci.nih.gov). The goal of this multi-institutional project is to identify and assess important genetic transcriptional changes in human brain, lung and ovarian cancers through genome analysis. The BCGC is a collaboration between Berkeley Lab, the University of California at Berkeley, and the University of California at San Francisco.
In the News: http://www.lbl.gov/Science-Articles/Archive/LSD-tumor-genomics.html

The Cancer Genome Atlas Research Network; Lawrence Berkeley National Laboratory contributors: Spellman PT, Purdom E, Jakkula LR, Lapuk AV, Marr H, Dorton S, Gi Choi Y, Han J, Ray A, Wang V, Durinck S, Robinson M, Wang NJ, Vranizan K, Peng V, Van Name E, Fontenay GV, Ngai J, Conboy JG, Parvin B, Feiler HS, Speed TP, Gray JW; Nature. 2008 Oct 23;455(7216):1061-8. PMID: 18772890.
CG, 10/08

Is 100 the New 80? Centenarians Are Studied to Find the Secret to Longevity



Centenarians may help researchers find the key to living longer, healthier lives. The reason, say scientists: centenarians may possess genes that protect them from disease into old age. The goal now is "to find the subtle genetic differences between individuals in the genes or families of genes associated with longevity," says **Judith Campisi**, with Berkeley Lab's Life Sciences Division. By understanding the underlying biology of aging, she notes, it may be possible to develop drugs that will delay age-related diseases such as some cancers, arthritis, diabetes, high blood pressure and heart disease.

[More info] http://www.sciam.com/article.cfm?id=centarians-studied-to-find-the-secret-of-longevity
Today at Berkeley Lab, 10/31/08

Glaeser and Nogales Organize HHMI Workshop on Electron Microscopy

Robert Glaeser and **Eva Nogales** were the organizers of the 2008 Workshop on Electron Microscopy held at the Howard Hughes Medical Institute's (HHMI) research campus, Janelia Farm, September, 23-24, 2008. The meeting, sponsored by HHMI, brought together leaders in the field of molecular structure studies by cryo-electron microscopy to identify the rate limiting factors in their research and discuss how to focus efforts on solving these problems. A consensus report (available at http://cryoem.berkeley.edu/janelia/) describes four principal areas that could be particularly productive: improvements that can be gained by development of an in-focus, phase-contrast device for electron microscopes; reduction of beam-induced effects, including specimen damage and charging which cause loss of resolution; improving the performance of imaging detectors, particularly with the application of modern integrated circuit technologies; development of aberration correctors, along with methods for interpretation of the mixed contrast mechanisms that contribute to images of thicker specimens such as encountered in electron tomography. All of these areas are under development in various projects at Berkeley Lab. Among other attendees from Berkeley Lab were **Ken Downing** and Peter Denes (Engineering Division). Workshop information and presentations are also available at http://cryoem.berkeley.edu/janelia/
Kenneth Downing, 10/08

Move from Building 74 in Progress

After many months of planning, the division has commenced moving the laboratories and offices from Building 74 to accommodate earthquake retrofit work and subsequent renovation of the building. Building 74 is expected to be vacated by January 2009. During the construction period research will be conducted in other life sciences buildings.
Damir Sudar, 10/08

Construction New Vivarium Completed

The construction of a new small animal vivarium has been completed and ACF manager **Randy DeGuzman** and his staff, with the help of responsible research staff, have commenced moving into the new facility. The move from Building 74, long the site of the vivarium, came just in time for the Building 74 evacuation to accommodate earthquake retrofit work. The move is going smoothly and is expected to be completed within the next few weeks. DeGuzman has employed extraordinary measures to ensure that the facility is ready for occupancy. The new facility offers improved air flow, lighting, and barrier controls and is equipped with a new cage washer and autoclave.
CG, 10/08

Awards

Celniker Promoted to Senior Scientist

Life sciences Staff Scientist **Susan Celniker**, a leader in the field of genomics, has been promoted to Senior Scientist. Her promotion was approved by the Lab Director after review of her promotion recommendation by the Division Staff Committee, **Joe Gray**, and the Laboratory Staff Committee. Celniker received her Ph.D. from the Department of Biochemistry, University of North Carolina, Chapel Hill, and has since made exceptional contributions to the understanding of Drosophila genome sequences and their functions. As an independent scientist, she has a remarkable publication record, grant portfolio and international reputation that reflect her significant contributions and standing in the community of scientists. Celniker's publication record includes the following high profile publications: Hoskins et al., Sequence finishing and mapping of Drosophila melanogaster heterochromatin. *Science*. 2007 Jun 15;316(5831):1625-8 and Stark et al., Discovery of functional elements in 12 Drosophila genomes using evolutionary signatures. *Nature*. 2007 Nov 8;450(7167):219-32.

Susan Celniker

CG, 10/08

Recent publications (selected)

Wilmes P, **Remis JP**, Hwang M, **Auer M**, Thelen MP, Banfield JF. Natural acidophilic biofilm communities reflect distinct organismal and functional organization. *The ISME Journal*, 2008 Oct 9. [Epub ahead of print]

> Pellicle biofilms colonize the air-solution interface of underground acid mine drainage (AMD) streams and pools within the Richmond Mine (Iron Mountain, Redding, CA, USA). They exhibit relatively low species richness and, consequently, represent good model systems to study natural microbial community structure. Fluorescence in situ hybridization combined with epifluorescent microscopy and transmission electron microscopy revealed spatially and temporally defined microbial assemblages. Leptospirillum group II dominates the earliest developmental stages of stream pellicles. With increasing biofilm maturity, the proportion of archaea increases in conjunction with the appearance of eukaryotes. In contrast, mature pool pellicles are stratified with a densely packed bottom layer of Leptospirillum group II, a less dense top layer composed mainly of archaea and no eukarya. Immunohistochemical detection of Leptospirillum group II cytochrome 579 indicates a high abundance of this protein at the interface of the biofilm with the AMD solution. Consequently, community architecture, which most likely develops in response to chemical gradients across the biofilm, is reflected at the functional gene expression level.

Alcaraz J, Xu R, **Mori H**, Nelson CM, **Mroue R**, **Spencer VA**, **Brownfield D**, **Radisky DC**, Bustamante C, **Bissell MJ**. Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia. *The EMBO Journal*, 2008 Oct 9. [Epub ahead of print]. PMID: 18843297

> In the mammary gland, epithelial cells are embedded in a 'soft' environment and become functionally differentiated in culture when exposed to a laminin-rich extracellular matrix gel. Here, we define the processes by which mammary epithelial cells integrate biochemical and mechanical extracellular cues to maintain their differentiated phenotype. We used single cells cultured on top of gels in conditions permissive for beta-casein expression using atomic force microscopy to measure the elasticity of the cells and their underlying substrata. We found that maintenance of beta-casein expression required both laminin signalling and a 'soft' extracellular matrix, as is the case in normal tissues in vivo, and biomimetic intracellular elasticity, as is the case in primary mammary epithelial organoids. Conversely, two hallmarks of breast cancer development, stiffening of the extracellular matrix and loss of laminin signalling, led to the loss of beta-casein expression and non-biomimetic intracellular elasticity. Our data indicate that tissue-specific gene expression is controlled by both the tissues' unique biochemical milieu and mechanical properties, processes involved in maintenance of tissue integrity and protection against tumorigenesis.

Busuttil RA, Muñoz DP, Garcia AM, Rodier F, Kim WH, Suh Y, Hasty P, **Campisi J**, Vijg J. Effect of Ku80 deficiency on mutation frequencies and spectra at a LacZ reporter locus in mouse tissues and cells. *PLoS ONE*, 2008;3(10):e3458. [Epub 2008 Oct 20]

> Non-homologous end joining (NHEJ) is thought to be an important mechanism for preventing the adverse effects of DNA double strand breaks (DSBs) and its absence has been associated with premature aging. To investigate the effect of inactivated NHEJ on spontaneous mutation frequencies and spectra in vivo and in cultured cells, we crossed a Ku80-deficient mouse with mice harboring a lacZ-plasmid-based mutation reporter. We analyzed various organs and tissues, as well as cultured embryonic fibroblasts, for mutations at the lacZ locus. When comparing mutant with wild-type mice, we observed a significantly higher number of genome rearrangements in liver and spleen and a significantly lower number of point mutations in liver

and brain. The reduced point mutation frequency was not due to a decrease in small deletion mutations thought to be a hallmark of NHEJ, but could be a consequence of increased cellular responses to unrepaired DSBs. Indeed, we found a substantial increase in persistent 53BP1 and gammaH2AX DNA damage foci in Ku80-/- as compared to wild-type liver. Treatment of cultured Ku80-deficient or wild-type embryonic fibroblasts, either proliferating or quiescent, with hydrogen peroxide or bleomycin showed no differences in the number or type of induced genome rearrangements. However, after such treatment, Ku80-deficient cells did show an increased number of persistent DNA damage foci. These results indicate that Ku80-dependent repair of DNA damage is predominantly error-free with the effect of alternative more error-prone pathways creating genome rearrangements only detectable after extended periods of time, i.e., in young adult animals. The observed premature aging likely results from a combination of increased cellular senescence and an increased load of stable, genome rearrangements.

Morrison HA, Dionne H, Rusten TE, Brech A, Fisher WW, Pfeiffer BD, **Celniker SE**, Stenmark H, Bilder D. Regulation of early endosomal entry by the Drosophila tumor suppressors Rabenosyn and Vps45. *Molecular Biology of the Cell*, 2008 Oct;19(10):4167-76. PMID: 18685079

The small GTPase Rab5 has emerged as an important regulator of animal development, and it is essential for endocytic trafficking. However, the mechanisms that link Rab5 activation to cargo entry into early endosomes remain unclear. We show here that Drosophila Rabenosyn (Rbsn) is a Rab5 effector that bridges an interaction between Rab5 and the Sec1/Munc18-family protein Vps45, and we further identify the syntaxin Avalanche (Avl) as a target for Vps45 activity. Rbsn and Vps45, like Avl and Rab5, are specifically localized to early endosomes and are required for endocytosis. Ultrastructural analysis of rbsn, Vps45, avl, and Rab5 null mutant cells, which show identical defects, demonstrates that all four proteins are required for vesicle fusion to form early endosomes. These defects lead to loss of epithelial polarity in mutant tissues, which overproliferate to form neoplastic tumors. This work represents the first characterization of a Rab5 effector as a tumor suppressor, and it provides in vivo evidence for a Rbsn-Vps45 complex on early endosomes that links Rab5 to the SNARE fusion machinery.

An X, Gauthier E, Zhang X, Guo X, Anstee DJ, Mohandas N, **Chasis JA**. Adhesive activity of Lu glycoproteins is regulated by interaction with spectrin. *Blood*, 2008 Sep 26. [Epub ahead of print] PMID: 18815288

The Lutheran (Lu) and Lu(v13) blood group glycoproteins function as receptors for extracellular matrix laminins. Lu and Lu(v13) are linked to the erythrocyte cytoskeleton through a direct interaction with spectrin. However, neither the molecular basis of the interaction nor its functional consequences have previously been delineated. In the present study, we defined the binding motifs of Lu and Lu(v13) on spectrin and identified a functional role for this interaction. We found that the cytoplasmic domains of both Lu and Lu(v13) bound to repeat 4 of the alpha spectrin chain. The interaction of full-length spectrin dimer to Lu and Lu(v13) was inhibited by repeat 4 of alpha spectrin. Further, resealing of this repeat peptide into erythrocytes led to weakened Lu-cytoskeleton interaction as demonstrated by increased detergent extractability of Lu. Importantly, disruption of the Lu-spectrin linkage was accompanied by enhanced cell adhesion to laminin. We conclude that the interaction of the Lu cytoplasmic tail with the cytoskeleton regulates its adhesive receptor function.

Maxwell CA, Fleisch MC, **Costes SV**, Erickson AC, Boissière A, Gupta R, **Ravani SA**, **Parvin B**, Barcellos-Hoff MH. Targeted and nontargeted effects of ionizing radiation that impact genomic instability. *Cancer Research*, 2008 Oct 15;68(20):8304-11. PMID: 18922902

Radiation-induced genomic instability, in which the progeny of irradiated cells display a high frequency of nonclonal genomic damage, occurs at a frequency inconsistent with mutation. We investigated the

mechanism of this nontargeted effect in human mammary epithelial cells (HMEC) exposed to low doses of radiation. We identified a centrosome-associated expression signature in irradiated HMEC and show here that centrosome deregulation occurs in the first cell cycle after irradiation, is dose dependent, and that viable daughters of these cells are genomically unstable as evidenced by spontaneous DNA damage, tetraploidy, and aneuploidy. Clonal analysis of genomic instability showed a threshold of >10 cGy. Treatment with transforming growth factor beta1 (TGFbeta), which is implicated in regulation of genomic stability and is activated by radiation, reduced both the centrosome expression signature and centrosome aberrations in irradiated HMEC. Furthermore, TGFbeta inhibition significantly increased centrosome aberration frequency, tetraploidy, and aneuploidy in nonirradiated HMEC. Rather than preventing radiation-induced or spontaneous centrosome aberrations, TGFbeta selectively deleted unstable cells via p53-dependent apoptosis. Together, these studies show that radiation deregulates centrosome stability, which underlies genomic instability in normal human epithelial cells, and that this can be opposed by radiation-induced TGFbeta signaling.

Hsieh MC, **Das D**, Sambandam N, Zhang MQ, Nahlé Z. Regulation of the PDK4 isozyme by the Rb-E2F1 complex. *The Journal of Biological Chemistry*, 2008 Oct 10;283(41):27410-7. PMID: 18667418

Loss of the transcription factor E2F1 elicits a complex metabolic phenotype in mice underscored by reduced adiposity and protection from high fat diet-induced diabetes. Here, we demonstrate that E2F1 directly regulates the gene encoding PDK4 (pyruvate dehydrogenase kinase 4), a key nutrient sensor and modulator of glucose homeostasis that is chronically elevated in obesity and diabetes and acutely induced under the metabolic stress of starvation or fasting. We show that loss of E2F1 in vivo blunts PDK4 expression and improves myocardial glucose oxidation. The absence of E2F1 also corresponds to lower blood glucose levels, improved plasma lipid profile, and increased sensitivity to insulin stimulation. Consistently, enforced E2F1 expression up-regulates PDK4 levels and suppresses glucose oxidation in C(2)C(12) myoblasts. Furthermore, inactivation of Rb, the repressor of E2F-dependent transcription, markedly induces PDK4 and triggers the enrichment of E2F1 occupancy onto the PDK4 promoter as detected by chromatin immunoprecipitation analysis. Two overlapping E2F binding sites were identified on this promoter. Transactivation assays later verified E2F1 responsiveness of this promoter element in C(2)C(12) myoblasts and IMR90 fibroblasts, an effect that was completely abrogated following mutation of the E2F sites. Taken together, our data illustrate how the E2F1 mitogen directly regulates PDK4 levels and influences cellular bioenergetics, namely mitochondrial glucose oxidation. These results are relevant to the pathophysiology of chronic diseases like obesity and diabetes, where PDK4 is dysregulated and could have implications pertinent to the etiology of tumor metabolism, especially in cancers with Rb pathway defects.

Martinez-Perez E, Schvarzstein M, Barroso C, Lightfoot J, **Dernburg AF**, Villeneuve AM. Crossovers trigger a remodeling of meiotic chromosome axis composition that is linked to two-step loss of sister chromatid cohesion. *Genes and Development*, 2008 Oct 15;22(20):2886-901. PMID: 18923085

Segregation of homologous chromosomes during meiosis depends on linkages (chiasmata) created by crossovers and on selective release of a subset of sister chromatid cohesion at anaphase I. During Caenorhabditis elegans meiosis, each chromosome pair forms a single crossover, and the position of this event determines which chromosomal regions will undergo cohesion release at anaphase I. Here we provide insight into the basis of this coupling by uncovering a large-scale regional change in chromosome axis composition that is triggered by crossovers. We show that axial element components HTP-1 and HTP-2 are removed during late pachytene, in a crossover-dependent manner, from the regions that will later be targeted for anaphase I cohesion release. We demonstrate correspondence in position and number between chiasmata and HTP-1/2-depleted regions and provide evidence that HTP-1/2 depletion boundaries mark crossover sites. In htp-1 mutants, diakinesis bivalents lack normal asymmetrical features,

and sister chromatid cohesion is prematurely lost during the meiotic divisions. We conclude that HTP-1 is central to the mechanism linking crossovers with late-prophase bivalent differentiation and defines the domains where cohesion will be protected until meiosis II. Further, we discuss parallels between the pattern of HTP-1/2 removal in response to crossovers and the phenomenon of crossover interference.

Comolli LR, Baker BJ, **Downing KH**, Siegerist CE, **Banfield JF**. Three-dimensional analysis of the structure and ecology of a novel, ultra-small archaeon. *The ISME Journal*, 2008 Oct 23. [Epub ahead of print] PMID: 18946497

Fully understanding the biology of acid mine drainage (AMD) is central to our ability to control and manipulate its environmental impact. Although genomics and biogeochemical methods are relatively well established in the field, their combination with high-resolution imaging of intact members of microbial biofilm communities has not yet reached its full potential. Here, we used three-dimensional (3D) cryogenic electron tomography to determine the size and ultrastructure of intact ARMAN cells, a novel ultra-small archaeon, and sought evidence for their interactions with other members of its community. Within acid mine drainage biofilms, apparently free-living ARMAN cells from a deeply branched archaeal lineage have volumes of 0.009-0.04 mum(3) (mean approximately 0.03+/-0.01 mum(3)), only approximately 92 ribosomes, yet are frequent hosts for replicating viruses. Organization within the periplasm and partitioning of ribosomes to the inner surface of the cytoplasmic membrane may be factors in size minimization. Most cells contain enigmatic tubular structures of unknown function. The low ribosome copy number per unit volume, indicative of slow growth rates and targeting of cells by diverse viruses may account for the low abundance of ARMAN cells compared with other biofilm community members. Our results provide the first 3D analysis of structural features of these novel and enigmatic cells and their interactions with at least two types of viruses. Our findings also emphasize that new biological phenomena remain to be discovered among lower abundance organisms from novel uncultivated lineages.The ISME Journal advance online publication, 23 October 2008; doi:10.1038/ismej.2008.99.

Srivastava S, **Gray JW**, Reid BJ, Grad O, Greenwood A, Hawk ET; Translational Research Working Group. Translational research working group developmental pathway for biospecimen-based assessment modalities. *Clinical cancer research: an official journal of the American Association for Cancer Research*, 2008 Sep 15;14(18):5672-7. PMID: 18794074

The Translational Research Working Group (TRWG) was created as a national initiative to evaluate the current status of National Cancer Institute's investment in translational research and envision its future. The TRWG conceptualized translational research as a set of six developmental processes or pathways focused on various clinical goals. One of those pathways describes the development of biospecimen-based assays that use biomarkers for the detection, diagnosis, and prognosis of cancer and the assessment of response to cancer treatment. The biospecimen-based assessment modality pathway was conceived not as comprehensive description of the corresponding real-world processes but rather as a tool designed to facilitate movement of a candidate assay through the translational process to the point where it can be handed off for definitive clinical testing. This paper introduces the pathway in the context of prior work and discusses key challenges associated with the biomarker development process in light of the pathway.

Sun Y, Wong N, Guan Y, Salamanca CM, Cheng JC, Lee JM, **Gray JW**, Auersperg N. The eukaryotic translation elongation factor eEF1A2 induces neoplastic properties and mediates tumorigenic effects of ZNF217 in precursor cells of human ovarian carcinomas. *International Journal of Cancer*, 2008 Oct 15;123(8):1761-9. PMID: 18661515

Ovarian epithelial carcinomas (OECs) frequently exhibit amplifications at the 20q13 locus which is the site of several oncogenes, including the eukaryotic elongation factor EEF1A2 and the transcription factor ZNF217. We reported previously that overexpressed ZNF217 induces neoplastic characteristics in precursor cells of OEC. Unexpectedly, ZNF217, which is a transcriptional repressor, enhanced expression of eEF1A2. In our study, array comparative genomic hybridization, single nucleotide polymorphism and Affymetrix analysis of ZNF217-overexpressing cell lines confirmed consistently increased expression of eEF1A2 but not of other oncogenes, and revealed early changes in EEF1A2 gene copy numbers and increased expression at crisis during immortalization. We defined the influence of eEF1A2 overexpression on immortalized ovarian surface epithelial cells, and investigated interrelationships between effects of ZNF217 and eEF1A2 on cellular phenotypes. Lentivirally induced eEF1A2 overexpression caused delayed crisis, apoptosis resistance and increases in serum-independence, saturation densities and anchorage independence. siRNA to eEF1A2 reversed apoptosis resistance and reduced anchorage independence in eEF1A2-overexpressing lines. Remarkably, siRNA to eEF1A2 was equally efficient in inhibiting both anchorage independence and resistance to apoptosis conferred by ZNF217 overexpression. Our data define neoplastic properties that are caused by eEF1A2 in nontumorigenic ovarian cancer precursor cells, and suggest that eEF1A2 plays a role in mediating ZNF217-induced neoplastic progression.

Mao JH, Kim IJ, Wu D, Climent J, Kang HC, DelRosario R, Balmain A. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science,* 2008 Sep 12;321(5895):1499-502. PMID: 18787170

The enzyme mTOR (mammalian target of rapamycin) is a major target for therapeutic intervention to treat many human diseases, including cancer, but very little is known about the processes that control levels of mTOR protein. Here, we show that mTOR is targeted for ubiquitination and consequent degradation by binding to the tumor suppressor protein FBXW7. Human breast cancer cell lines and primary tumors showed a reciprocal relation between loss of FBXW7 and deletion or mutation of PTEN (phosphatase and tensin homolog), which also activates mTOR. Tumor cell lines harboring deletions or mutations in FBXW7 are particularly sensitive to rapamycin treatment, which suggests that loss of FBXW7 may be a biomarker for human cancers susceptible to treatment with inhibitors of the mTOR pathway.

Marchetti F, Bishop J, Lowe X, **Wyrobek AJ**. Chromosomal mosaicism in mouse to-cell embryos after paternal exposure to acrylamide. *Toxicological Sciences : an official journal of the Society of Toxicology,* 2008 Oct 16. [Epub ahead of print] PMID: 18930949

Chromosomal mosaicism in human preimplantation embryos is a common cause of spontaneous abortions, however, our knowledge of its etiology is limited. We used multicolor fluorescence in situ hybridization (FISH) painting to investigate whether paternally-transmitted chromosomal aberrations result in mosaicism in mouse 2-cell embryos. Paternal exposure to acrylamide, an important industrial chemical also found in tobacco smoke and generated during the cooking process of starchy foods, produced significant increases in chromosomally defective 2-cell embryos, however, the effects were transient primarily affecting the postmeiotic stages of spermatogenesis. Comparisons with our previous study of zygotes demonstrated similar frequencies of chromosomally abnormal zygotes and 2-cell embryos suggesting that there was no apparent selection against numerical or structural chromosomal aberrations. However, the majority of affected 2-cell embryos were mosaics showing different chromosomal abnormalities in the two blastomeric metaphases. Analyses of chromosomal aberrations in zygotes and 2-cell embryos showed a tendency for loss of acentric fragments during the first mitotic division of embryogenesis, while both dicentrics and translocations apparently underwent proper segregation. These results suggest that embryonic development can proceed up to the end of the second cell cycle of development in the presence of abnormal paternal chromosomes and that even dicentrics can persist through cell division. The high incidence of chromosomally mosaic 2-cell embryos suggests

that the first mitotic division of embryogenesis is prone to missegregation errors and that paternally-transmitted chromosomal abnromalities increase the risk of missegregation leading to embryonic mosaicism.

Salo R, **Nordahl TE**, Buonocore MH, Natsuaki Y, Waters C, Moore CD, Galloway GP, Leamon MH. Cognitive control and white matter callosal microstructure in methamphetamine-dependent subjects: a diffusion tensor imaging study. *Biological Psychiatry*, 2008 Sep 22. [Epub ahead of print]

BACKGROUND: Methamphetamine (MA) abuse causes damage to structures within the human cerebrum, with particular susceptibility to white matter (WM). Abnormalities have been reported in anterior regions with less evidence of changes in posterior regions. Methamphetamine abusers have also shown deficits on attention tests that measure response conflict and cognitive control. METHODS: We examined cognitive control with a computerized measure of the Stroop selective attention task and indices of WM microstructure obtained from diffusion tensor imaging (DTI) in the callosal genu and splenium of 37 currently abstinent MA abusers and 17 non-substance abusing control subjects. Measurements of fractional anisotropy (FA), apparent diffusion coefficient (ADC) of callosal fibers, and diffusion tensor eigenvalues were obtained in all subjects. RESULTS: The MA abusers exhibited greater Stroop reaction time interference (i.e., reduced cognitive control) ($p = .04$) compared with control subjects. After correcting for multiple comparisons, FA within the genu correlated significantly with measures of cognitive control in the MA abusers ($p = .04$, Bonferroni corrected) but not in control subjects ($p = .26$). Group differences in genu but not splenium FA were trend significant ($p = .09$). CONCLUSIONS: Methamphetamine abuse seems to alter anterior callosal WM microstructure with less evidence of change within posterior callosal WM microstructure. The DTI indices within the genu but not splenium correlated with measures of cognitive control in chronic MA abusers.

Clarey MG, Botchan M, **Nogales E**. Single particle EM studies of the Drosophila melanogaster origin recognition complex and evidence for DNA wrapping. *Journal of Structural Biology*, 2008 Sep 11. [Epub ahead of print]

Hyperphosphorylation of the Drosophila melanogaster origin recognition complex (DmORC) by cyclin dependent kinases (CDKs) allows nucleotide binding but inhibits the ATPase activity of Orc1, and ablates the ATP-dependent interaction of ORC with DNA. Here we present single particle electron microscopy (EM) studies of ORC bound to nucleotide in both the dephosphorylated and hyper-phosphorylated states. 3D image reconstructions show that nucleotide binding gives rise to an analogous conformation independent of phosphorylation state. At the intermediate resolution achieved in our studies, ATP promotes changes along the toroidal core of the complex with negligible differences contributed by phosphorylation. Thus, hyperphosphorylation of DmORC does not induce meso-scale rearrangement of the ORC structure. To better understand ORC's role in origin remodeling, we performed atomic force microscopy (AFM) studies that show the contour length of a 688bp linear DNA fragment shortens by the equivalent of approximately 130bp upon ORC binding. This data, coupled with previous studies that showed a linking number change in circular DNA upon ORC binding, suggests that ORC may wrap the DNA in a manner akin to DnaA. Based on existing data and our structures, we propose a subunit arrangement for the AAA+ and winged helix domains, and in addition, speculate on a path of the 133bp of DNA around the ORC complex.

Hogart A, Leung KN, **Wang NJ**, Wu DJ, Driscoll J, Vallero RO, Schanen C, Lasalle JM. Chromosome 15q11-13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number. *Journal of Medical Genetics,* 2008 Oct 7. [Epub ahead of print] PMID: 18835857

BACKGROUND: Chromosome 15q11-13 contains a cluster of imprinted genes essential for normal mammalian neurodevelopment. Deficiencies in paternal or maternal 15q11-13 alleles result in Prader-Willi or Angelman syndromes, respectively, and maternal duplications lead to a distinct condition that often includes autism. Overexpression of maternally expressed imprinted genes is predicted to cause 15q11-13-associated autism, but a link between gene dosage and expression has not been experimentally determined in brain. METHODS: Post-mortem brain tissue was obtained from a male with 15q11-13 hexasomy and a female with 15q11-13 tetrasomy. Quantitative RT-PCR was used to measure ten 15q11-13 transcripts in maternal 15q11-13 duplication, Prader-Willi syndrome, and control brain samples. Southern blot, bisulfite sequencing and fluorescence in situ hybridization were used to investigate epigenetic mechanisms of gene regulation. RESULTS: Gene expression and DNA methylation correlated with parental gene dosage in the male 15q11-13 duplication sample with severe cognitive impairment and seizures. Strikingly, the female with autism and milder Prader-Willi-like characteristics demonstrated unexpected deficiencies in the paternally expressed transcripts SNRPN, NDN, HBII85, and HBII52 and unchanged levels of maternally expressed UBE3A compared to controls. Paternal expression abnormalities in the female duplication sample were consistent with elevated DNA methylation of the 15q11-13 imprinting control region (ICR). Expression of nonimprinted 15q11-13 GABA receptor subunit genes was significantly reduced specifically in the female 15q11-13 duplication brain without detectable GABRB3 methylation differences. CONCLUSION: Our findings suggest that genetic copy number changes combined with additional genetic or environmental influences on epigenetic mechanisms impact outcome and clinical heterogeneity of 15q11-13 duplication syndromes.

Bhalla N, **Wynne DJ**, Jantsch V, **Dernburg AF**. ZHP-3 acts at crossovers to couple meiotic recombination with synaptonemal complex disassembly and bivalent formation in C. elegans. *PLoS Genetics*, 2008 Oct;4(10):e1000235. [Epub 2008 Oct 24] PMID: 18949042

Crossover recombination and the formation of chiasmata normally ensure the proper segregation of homologous chromosomes during the first meiotic division. zhp-3, the Caenorhabditis elegans ortholog of the budding yeast ZIP3 gene, is required for crossover recombination. We show that ZHP-3 protein localization is highly dynamic. At a key transition point in meiotic prophase, the protein shifts from along the length of the synaptonemal complex (SC) to an asymmetric localization on the SC and eventually becomes restricted to foci that mark crossover recombination events. A zhp-3::gfp transgene partially complements a null mutation and reveals a separation of function; although the fusion protein can promote nearly wild-type levels of recombination, aneuploidy among the progeny is high, indicating defects in meiotic chromosome segregation. The structure of bivalents is perturbed in this mutant, suggesting that the chromosome segregation defect results from an inability to properly remodel chromosomes in response to crossovers. smo-1 mutants exhibit phenotypes similar to zhp-3::gfp mutants at higher temperatures, and smo-1; zhp-3::gfp double mutants exhibit more severe meiotic defects than either single mutant, consistent with a role for SUMO in the process of SC disassembly and bivalent differentiation. We propose that coordination of crossover recombination with SC disassembly and bivalent formation reflects a conserved role of Zip3/ZHP-3 in coupling recombination with SC morphogenesis.